

ELECTRON MICROSCOPY WITH SUPERCONDUCTING LENSES

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The resolving power of the electron microscope has already enabled us to see directly structures of molecular dimensions, and the atomic spacing in crystalline lattices (1-3). Although the wavelength of electrons in standard microscopes is 100,000 times shorter than the wavelength of light, the performance of present electromagnetic or electrostatic lenses is limited by aberrations to usable apertures of 1.5 of the best light microscope lenses.

Correction of lens aberrations, stabilization of the lens excitation current and accelerating voltage together with improvement of electron source characteristics are, therefore, among the major instrumental problems which must be solved before the ultimate theoretical resolution of about 2 Ångströms is effectively attained. Since the focal length of a magnetic lens is dependent on the electron energy, as well as on the lens excitation current, the power supplies must consistently maintain a degree of stability of 1 to 2 parts per million for high resolution.

In addition to these critical demands straining the limits of conventional technology, it would be highly desirable to overcome the significant limitation imposed by the saturation of the iron pole pieces, thus making it possible to design "stronger" lenses of shorter focal length and correspondingly reduced aberrations.

As pointed out by Heidenreich, all of the perturbations that affect image quality and resolution introduce diffuseness into the object plane, and this uncertainty introduced by the objective lens is currently the major limiting factor in the resolution of atomic positions. Based on previous work in low-temperature electron microscopy (5, 6), the author has indicated the unique advantages to be derived from the use of superconducting electromagnetic lenses operating at

liquid helium temperatures, with regard to both instrumentation and specimen preservation. With the new high-field superconducting solenoids of niobium-tin and niobium-zirconium now available, it is possible to obtain large uniform magnetic fields which are highly homogeneous to better than 1 part in 10^6 to 10^7 and are extremely stable and noise-free, when the solenoids are operated at liquid helium temperatures short-circuited, or in the "persistent current mode" under appropriately controlled conditions (4).

As part of a comprehensive program carried out in the new electron microscope facility in the Department of Biophysics at the University of Chicago, we have performed the first successful electron microscopy experiments with high-field superconducting solenoid lenses (5). In a series of controlled experiments with a specially designed electron microscope using high-field superconducting niobium-zirconium solenoid lenses (supplied by the Westinghouse Cryogenics System Department) in an open-air-core, liquid helium Dewar (Figs. 1, 2), electron microscopic images of test specimens have been recorded (Fig. 3) while operating at 32,200 gauss in a persistent current mode with highly regulated accelerating potentials of 4 to 8 kV. These preliminary experiments demonstrated the exceptional stability of the images over periods of continuous operation of 4 to 8 hours, and the relatively high quality of the images.

During the past year, these experiments have been continued with different types of electron microscopes with superconducting solenoid lenses operating at 4 to 32 kilogauss in a persistent current mode, without pole pieces and with pole pieces of various kinds. Pointed filament sources and highly regulated accelerating potentials of 4, 6, 8 and 50 kV were used, taking special precautions to minimize mechanical, magnetic and electrical field perturbations. These experiments (7, 8) carried out at electron-optical magnifications of 50 to 4000 x using replicas of diffraction

Electron Microscopy with Superconducting Lenses.

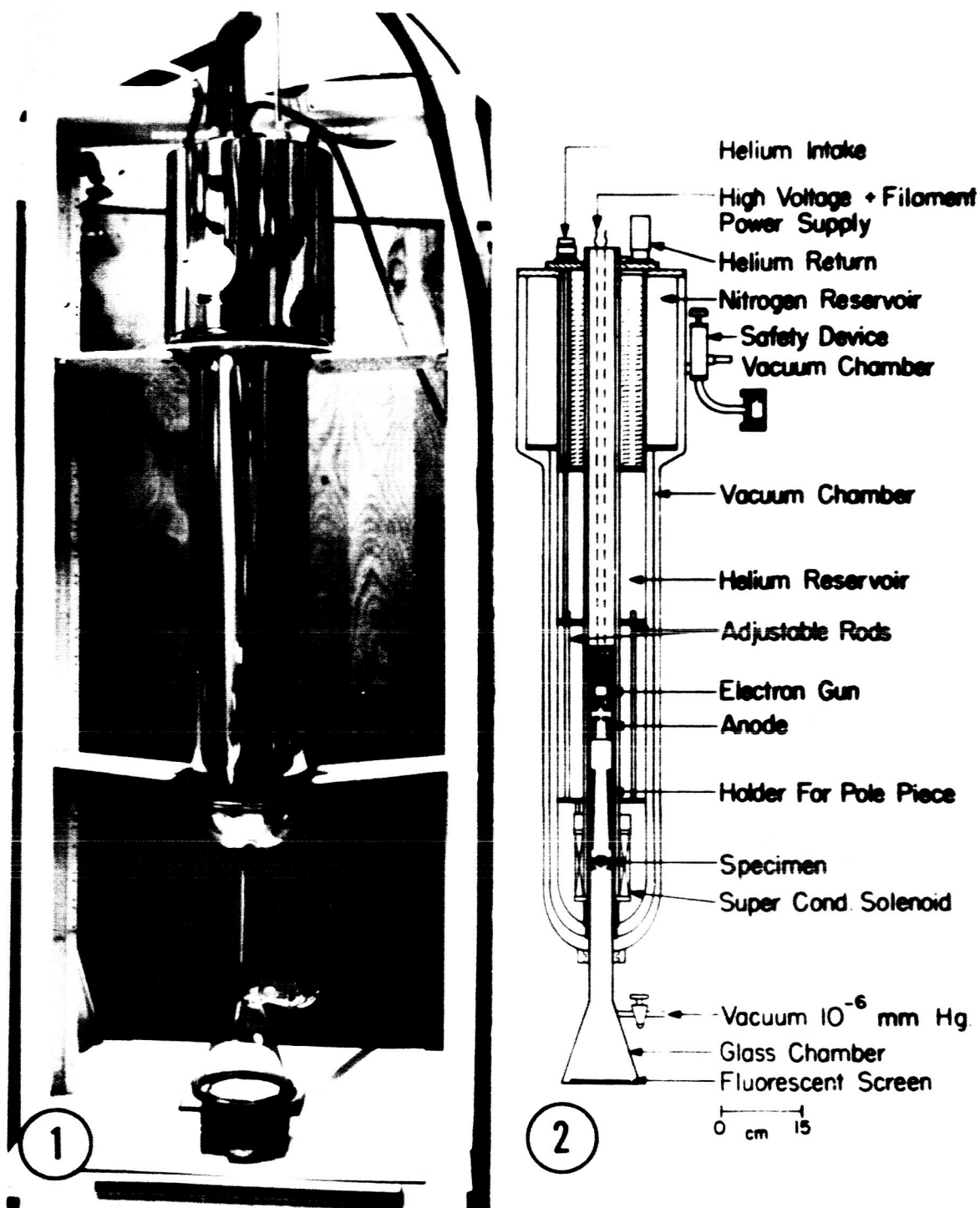


FIG. 1-Photograph, and (2) diagram of basic equipment for electron microscopy with high-field superconducting lens, comprising air-core liquid-helium Dewar with superconducting solenoid (operating at $\sim 2,200$ gauss in persistent current mode), and inserted electron microscope. Fluorescent screen attached to high vacuum photographic recording chamber not shown here. (From: H. Fernandez-Ortiz, Proc. Natl. Acad. Sciences, 53, 445, 1965.)

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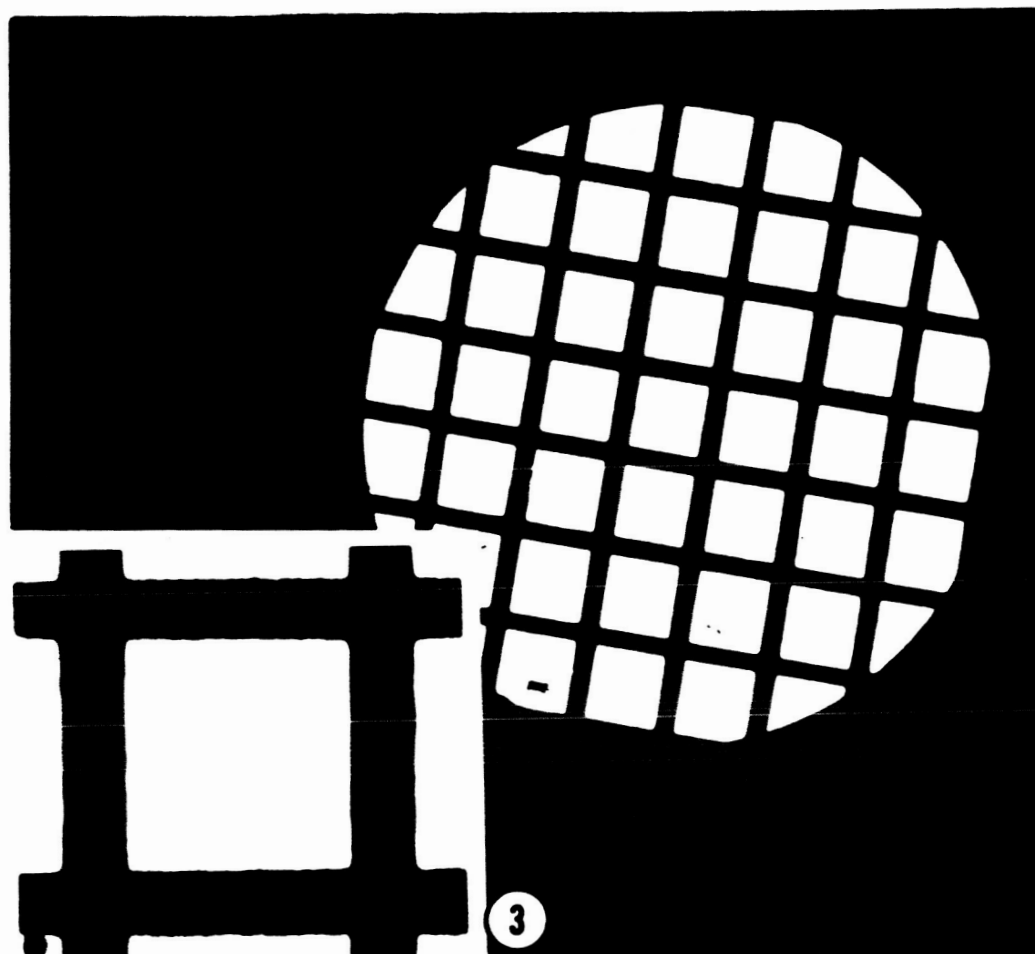


FIG. 3 (a)-Electron micrograph of 200 mesh copper specimen grid recorded directly on high resolution photographic plate with high-field superconducting lens (32,200 gauss in persistent current mode) in cryo-electron microscope without pole pieces. 6 kV accelerating potential. Original electron-optical magnification: 50 x. (b) Enlarged grid section: 210 x.

gratings as test specimens (Figs. 4, 5) have fully confirmed the exceptional stability of the images and their unimpaired high quality over extended periods. The images are focused on the screen with a precision current settability unit manufactured by Westinghouse Cryogenics Division. Once correct focus has been reached, the superconducting solenoid is simply placed in the persistent current mode, and the power supply removed. The images thus maintained without any external current source are of an unprecedented degree of stability, permitting exposures of 30 seconds to several minutes with microbeam illumination of low intensity (Figs. 4, 5) as compared with the normal exposure times of a few seconds only imposed by the lens instabilities in conventional microscopes under similar conditions.

As shown in Fig. 5 (S), fine detail is discernible in micrographs recorded with a single lens as a result of the integrated long-term exposure made possible by the high stability of the superconducting lens. In fact, this exceptional degree of stability has led to a critical survey and detection of other sources of mechanical, magnetic and electrical perturbations which had previously been masked by the lens fluctuations and ripple.

Preliminary experiments using superconducting lenses in combination with pointed filament sources appear promising for recording holograms according to the Gabor diffraction microscopy and wave reconstruction principle (9). Although there are still numerous experimental difficulties, these preliminary results and new observations on imaging phenomena with superconducting solenoid lenses are providing essential data for the design of new types of miniaturized cryo-electron microscopes immersed in a liquid helium cryostat (6, 7), and of a separate superconducting solenoid objective lens with appropriate pole pieces and stigmators which may be used in conjunction with modern high resolution electron microscopes to replace the objective lens.

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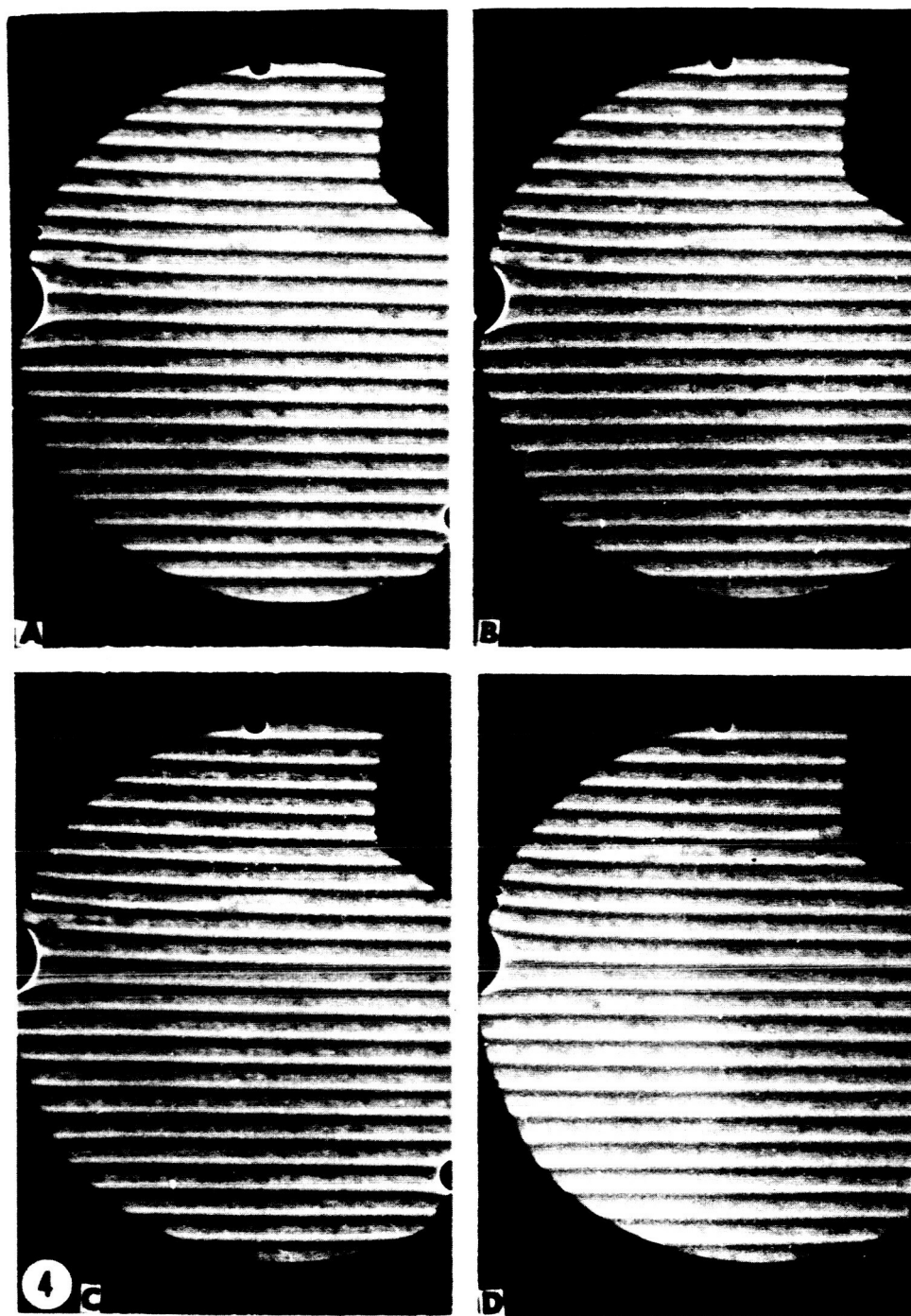


FIG. 4 (a-d)-Electron micrographs of 25,000 lines per inch diffraction grating replica recorded under same controlled conditions with high-field superconducting lens operating in persistent current mode continuously over a total period of 12 hours. These 4 micrographs (selected from the first and terminal series) were recorded directly on high resolution films at 5 to 15 minute intervals in cryo-electron microscope (50 kV) at electron optical magnification of 2000 x. They demonstrate exceptional degree of long-term stability attainable with superconducting lenses in persistent current mode.

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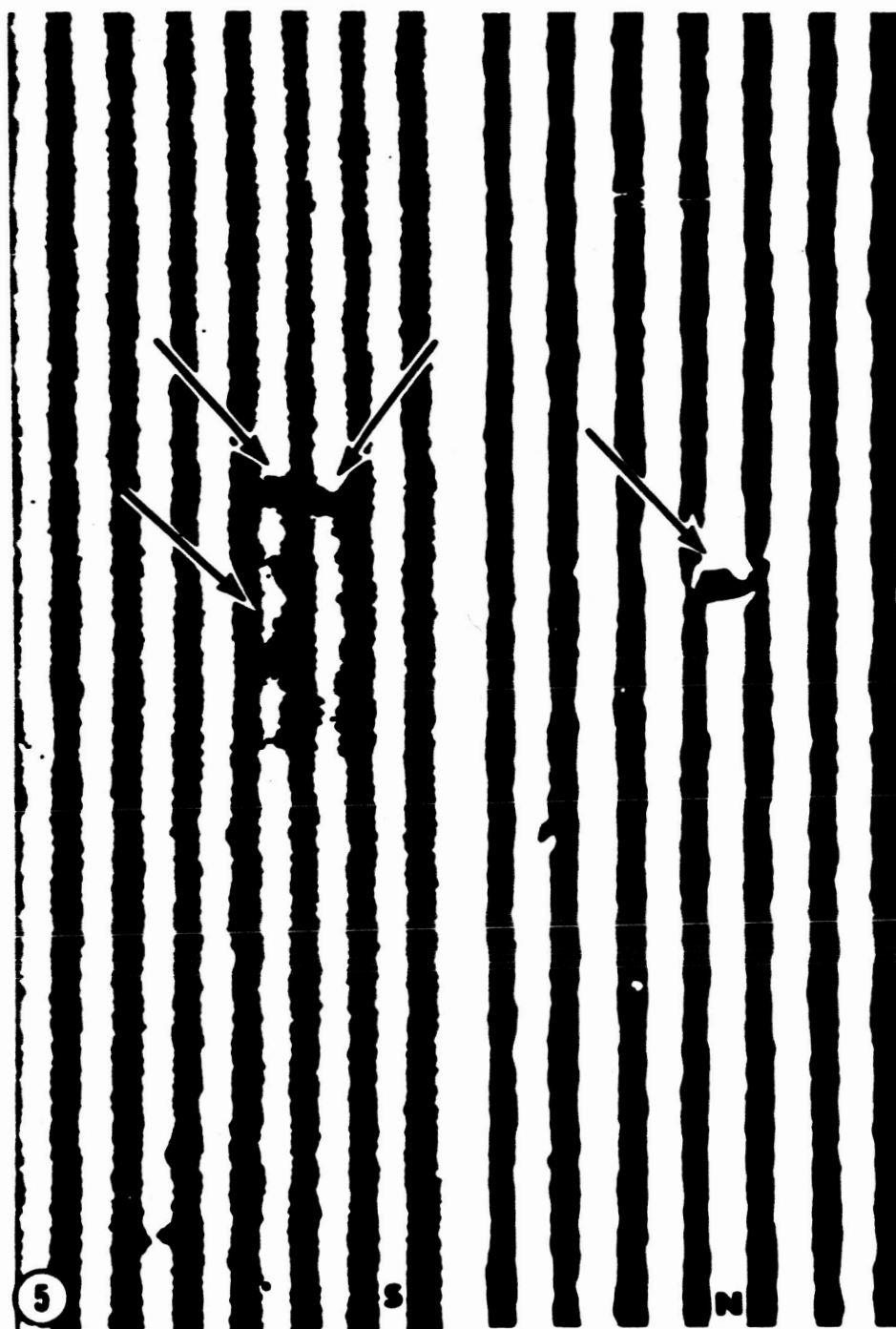


FIG. 5 (S) Electron micrograph of replica of 54,864 line per inch diffraction grating recorded with high-field superconducting lens in persistent current mode with pole piece at 50 kV, original electron optical magnification: X 220. Compare fine detail discernible in this micrograph as a result of the integrated long-term exposure possible due to high stability of superconducting lens, with corresponding control (N) which was recorded under identical conditions with same specimen using a commercial high resolution electron microscope with objective lens only. Fine structures seen in (S) are actually found when replica is examined at higher electron optical magnifications in standard electron microscopes.

As previously described (5-7), the desirability of examining specimens at liquid helium temperatures would represent in itself a major advantage for the biologist and physicist in pursuing this experimental approach. These "cryo-electron microscopes" operating at temperatures of 1° to 4° K would embody the following distinctive features: (a) highly stable superconducting electromagnetic lenses, either of the miniature type (thin film, or "trapped flux") without pole pieces, or with pole pieces of iron or possibly the rare earth metals dysprosium or holmium which have a higher saturation value. These lenses could be corrected by appropriate superconducting stigmators, focused and switched into the persistent current mode for maximum stability; (b) operation in the ultrahigh cryogenic vacuum at liquid helium temperatures resulting in decisive advantages of minimized specimen contamination, specimen damage, and thermal noise; (c) optimum conditions for both low-voltage (i.e., 1-10 kV) and high-voltage electron microscopy.

Additional use of high efficiency image viewing (single-crystal fluorescent screens), electronic image intensifiers and related devices would make it possible to use high-speed cinematography and stroboscopic recording for attainment of high temporal resolution combined with high spatial resolution. In principle, such a "cryo-electron microscope system" would also be an ideal device for controlled application of electron microbeams (ca. 50 to 500 Å diameter) of precisely defined duration and intensity for ultraminiaturization, storage of information, and in general, for controlled observation, irradiation and manipulation of hydrated biological systems at the molecular level under conditions of minimum perturbation.

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